*Unmethylated A vector*

| *replicative genes* | *Active copy of marker gene* | *X* |
| *Inactive copy of marker gene* | | *Y* |

*Methylated I Vector*

Anneal and ligate

*marker gene*

*test sequence*

*Heteroduplex DNA having one methylated strand*

Transform bacterial host
Plate transformants

*Perfect match between X and Y*

*Mismatch present between X and Y*

*Colonies express active marker gene*
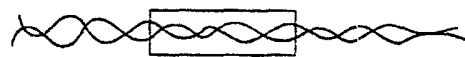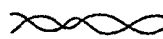
*Colonies lack active marker gene*

## FIG. 1

Genomic copy Y sequence

PCR amplification
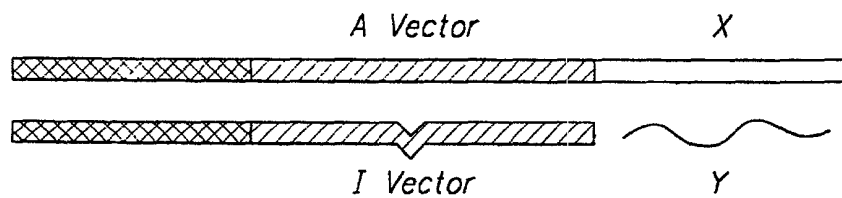
Denature

Hybridize vectors and Y strand.
Ligate.
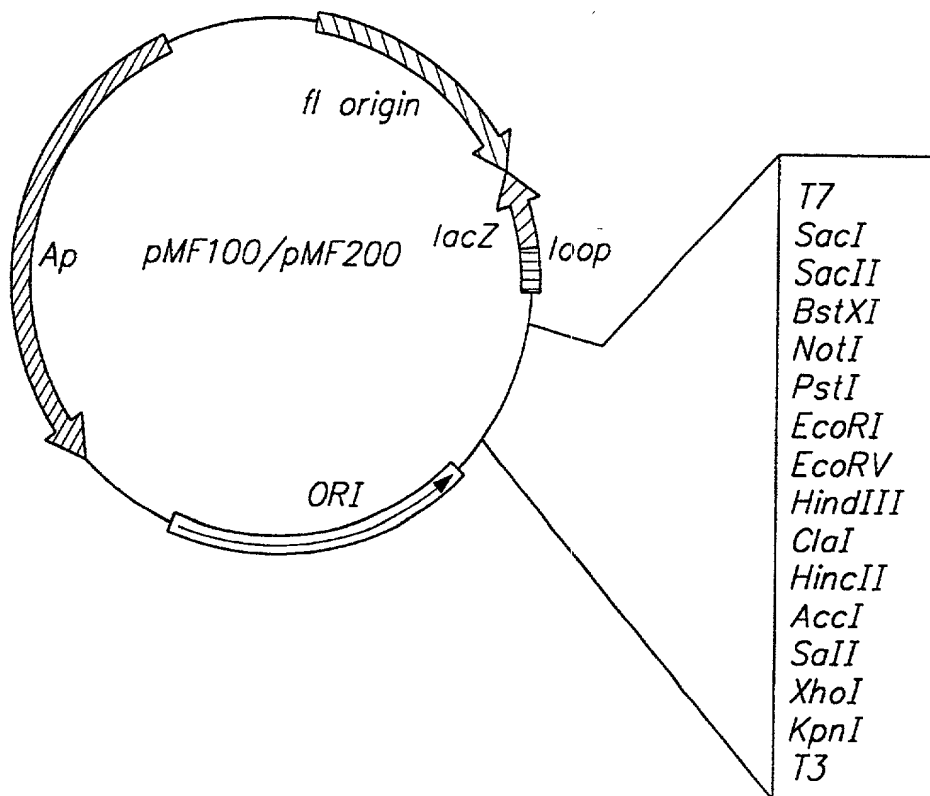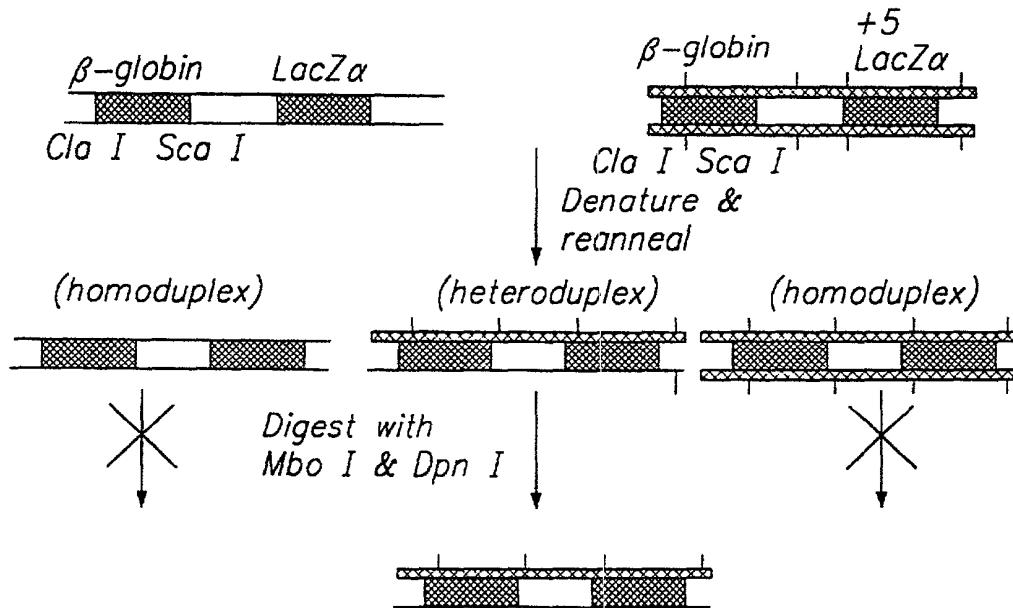
A Vector                    X

I Vector                    Y

FIG. 2

*fl origin*

*Ap*  *pMF100/pMF200*  *lacZ*  *loop*

*ORI*

T7
SacI
SacII
BstXI
NotI
PstI
EcoRI
EcoRV
HindIII
ClaI
HincII
AccI
SaII
XhoI
KpnI
T3

FIG. 3

FIG. 4

*pick blue colonies*

*Isolate DNA*

*pick white colonies*

*Isolate DNA*

*Use as a hybridization probe
on a dot blot filter containing
the fragments tested*

*Use as a hybridization probe
on a dot blot filter containing
the fragments tested*

*comparing the hybridization signal on both filters,
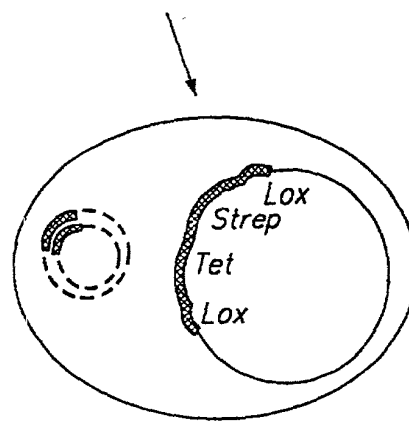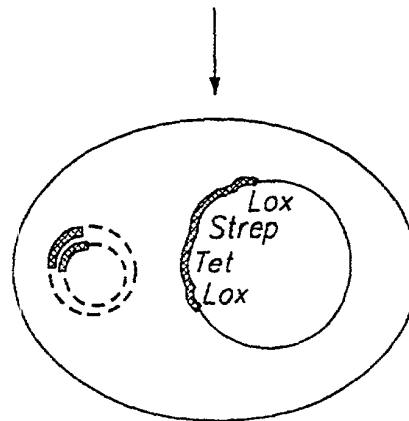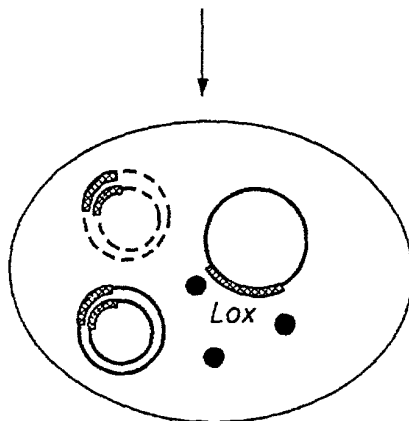one can determine which fragments are variant*
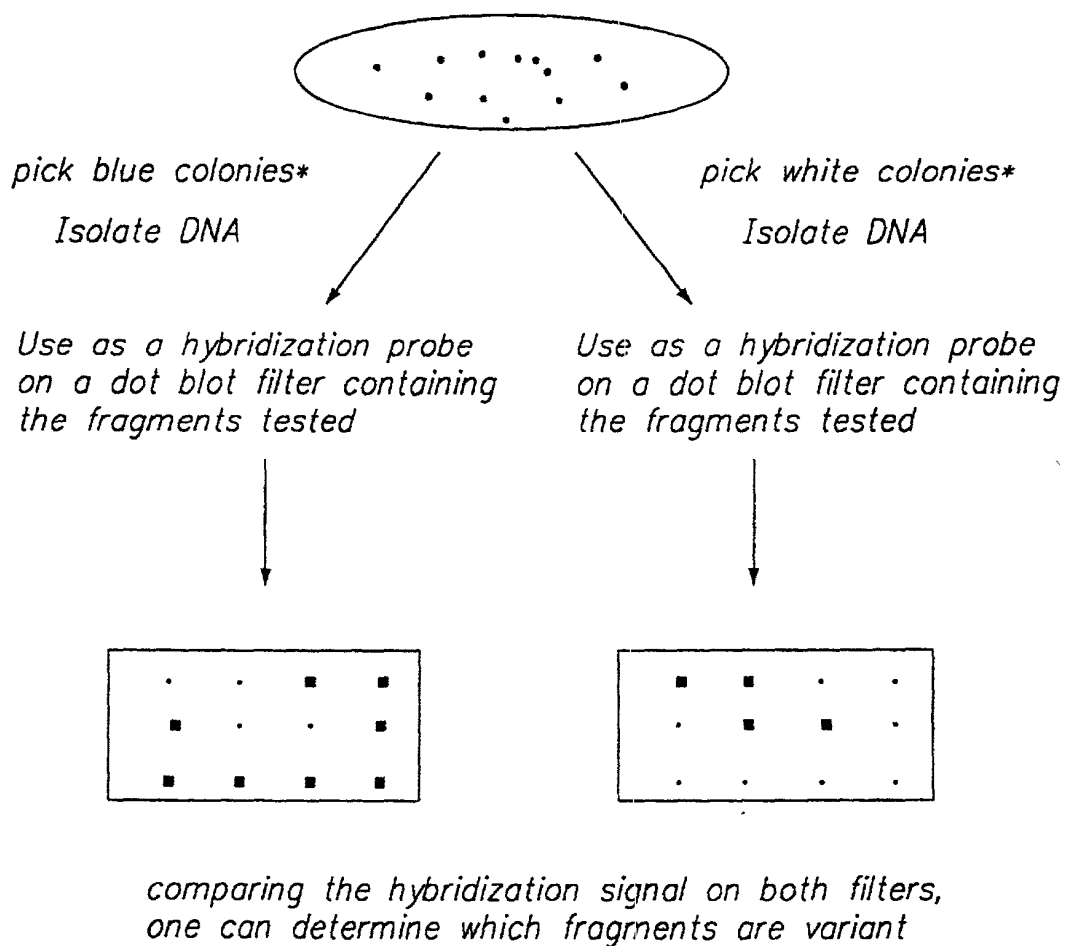
## FIG. 5
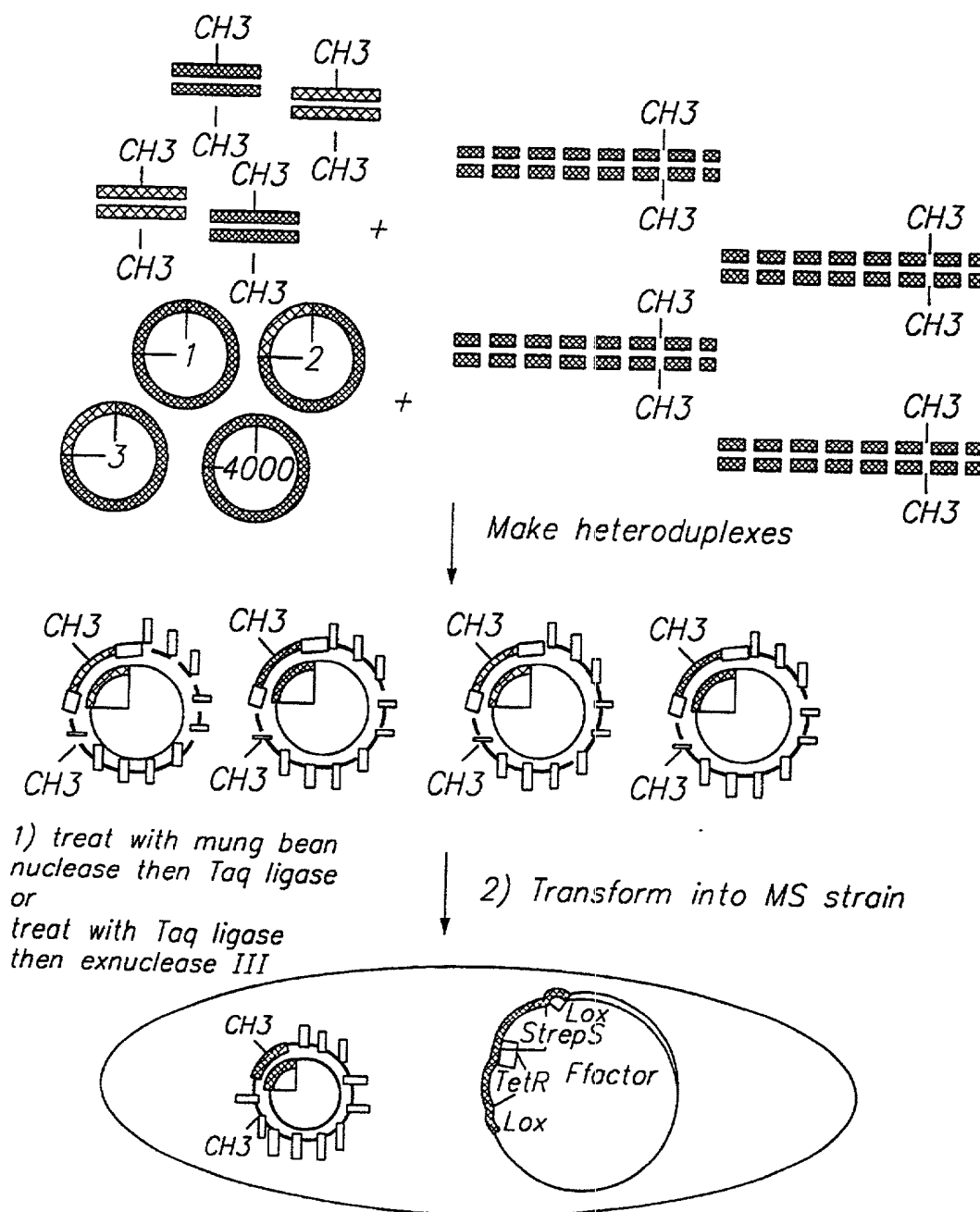
FIG. 6A
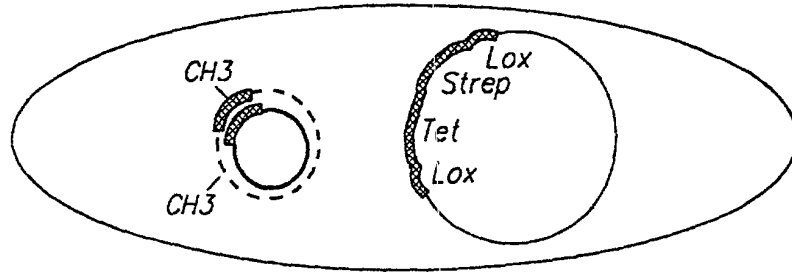
In absence of a variation,
no repair occurs.
Both strands are replicated

In presence of a variation,
repair occurs. Only the
strand w/inactive Cre is replicated
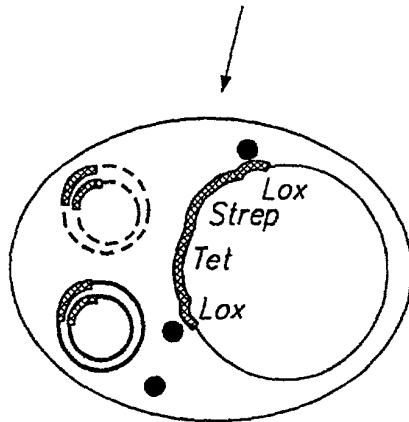
Active Cre is present in the cell

Active Cre is absent in the cell

Cell is Tet sensitive
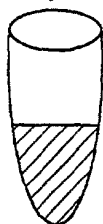& Strep resistant

Cell is Tet resistant
& Strep sensitive

FIG. 6B

*Cells are grown in two tubes supplemented either with*
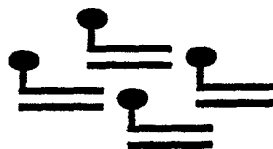
*Tetracycline        or        Streptomycin*



*Next day DNA is preped from the pool of the cells
grown in each tube*

*DNA from the Tet pool is
labeled with green fluorescence*

*DNA from the Strep pool is
labeled with red fluorescence*



*DNA from both pools are mixed and hybridized
to a DNA microarray.
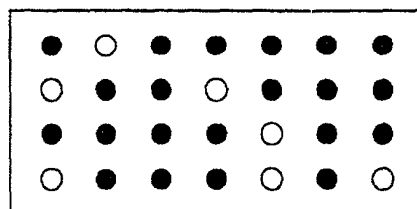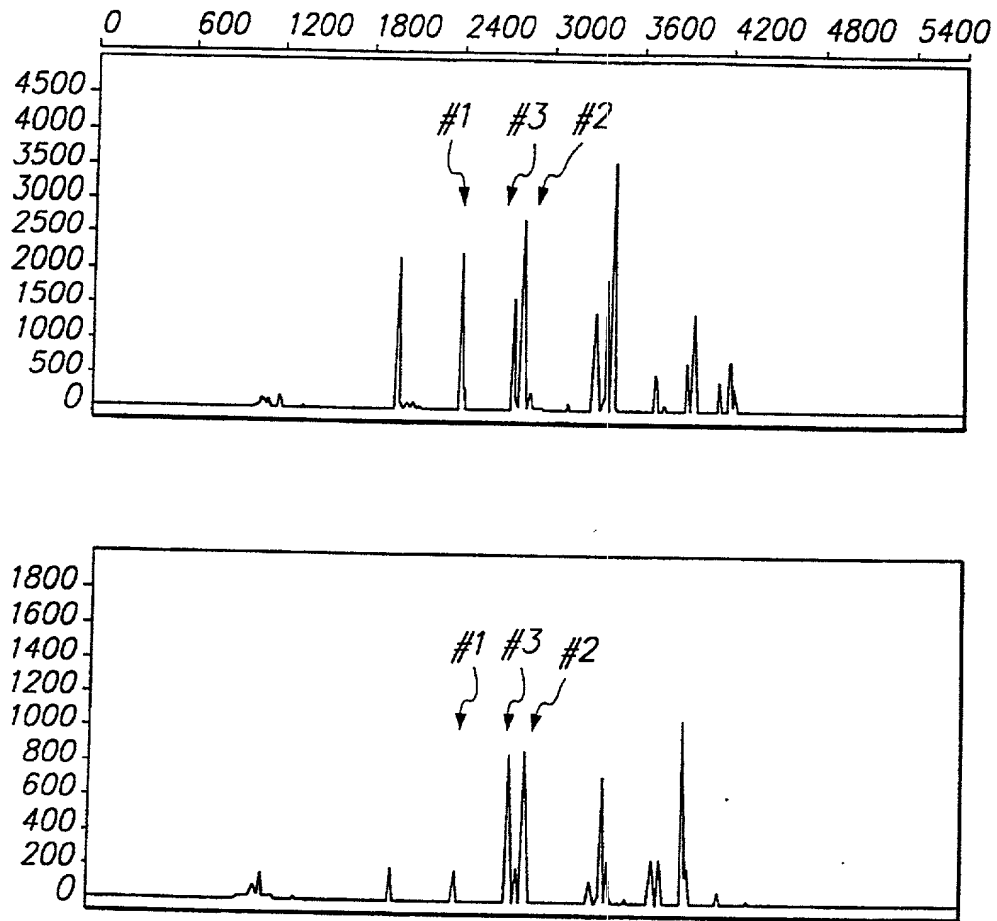Each spot corresponds to a different gene fragment
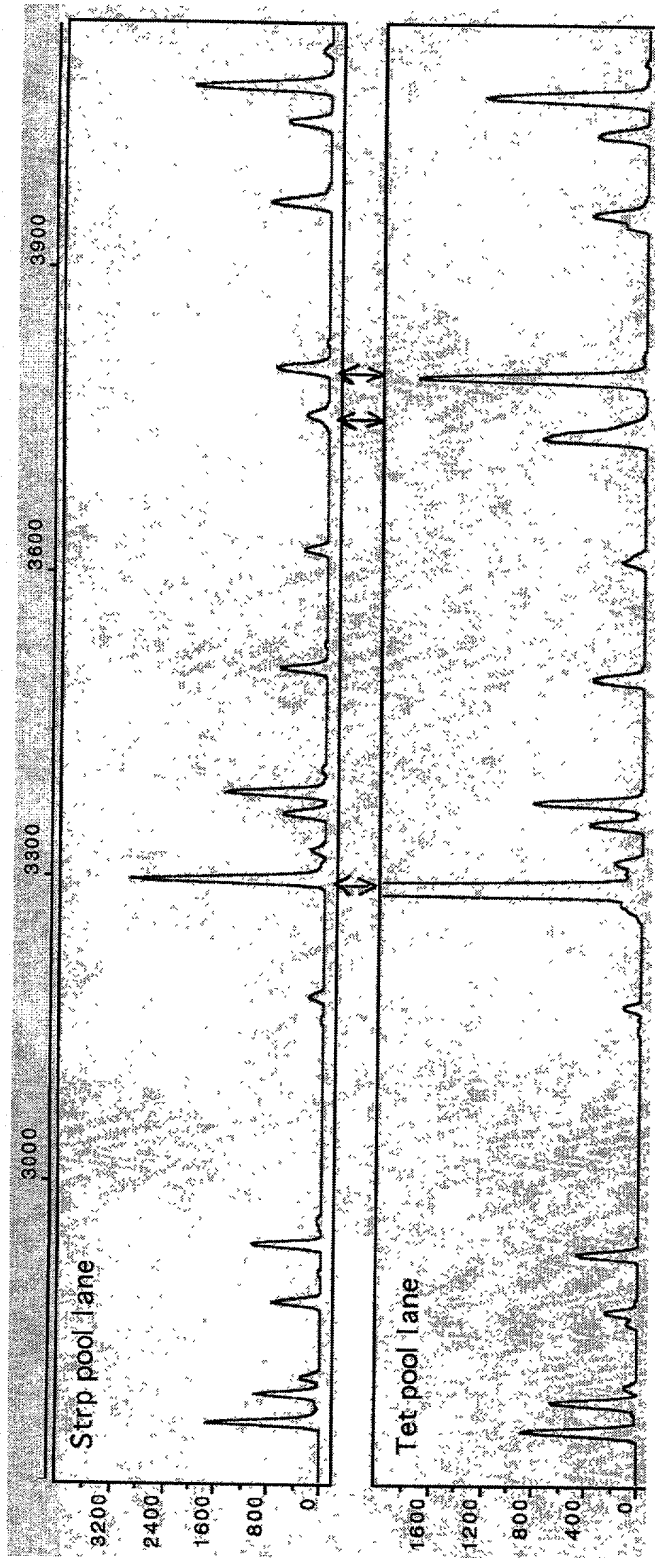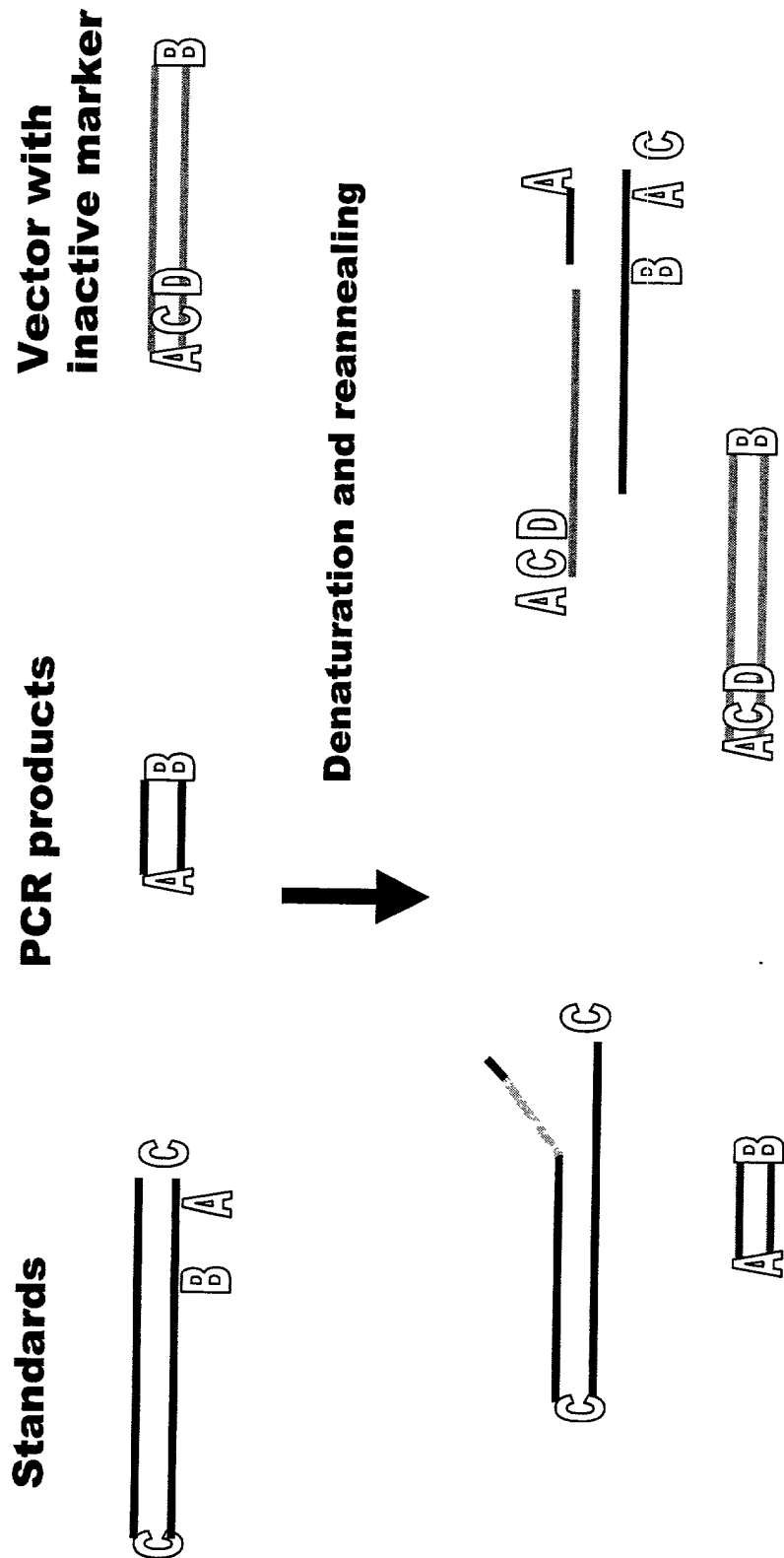that is being tested*



*FIG. 6C*

FIG. 7

FIG. 8

**FIG. 9A**

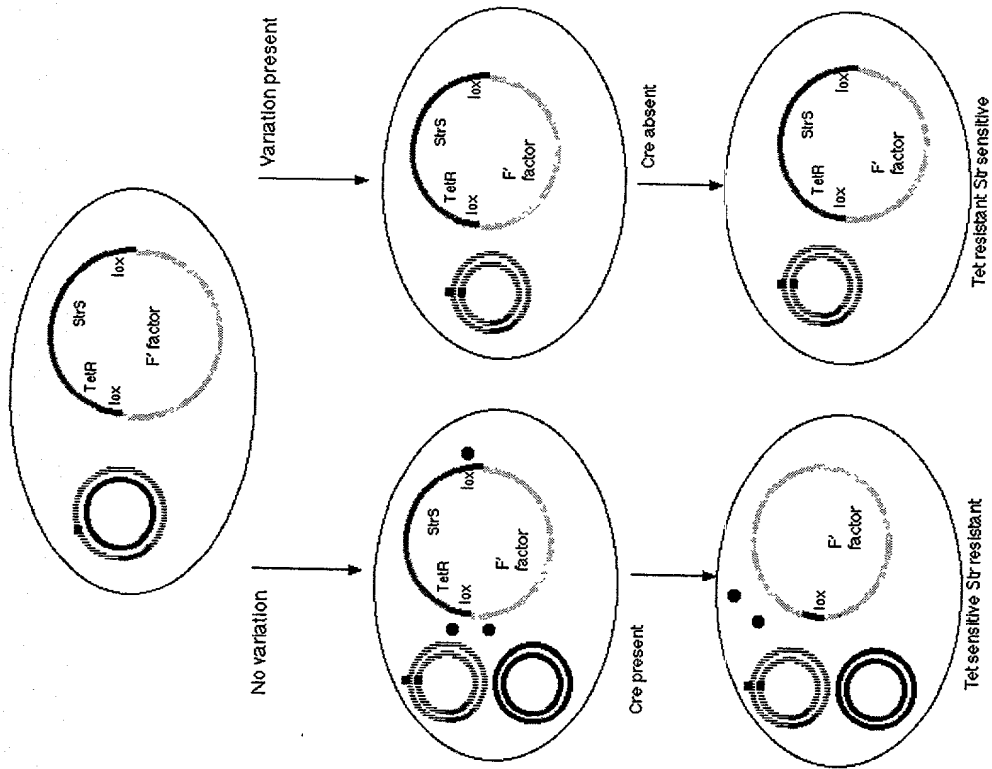elimination of wholly or partially single stranded species

ligation of compatible ends
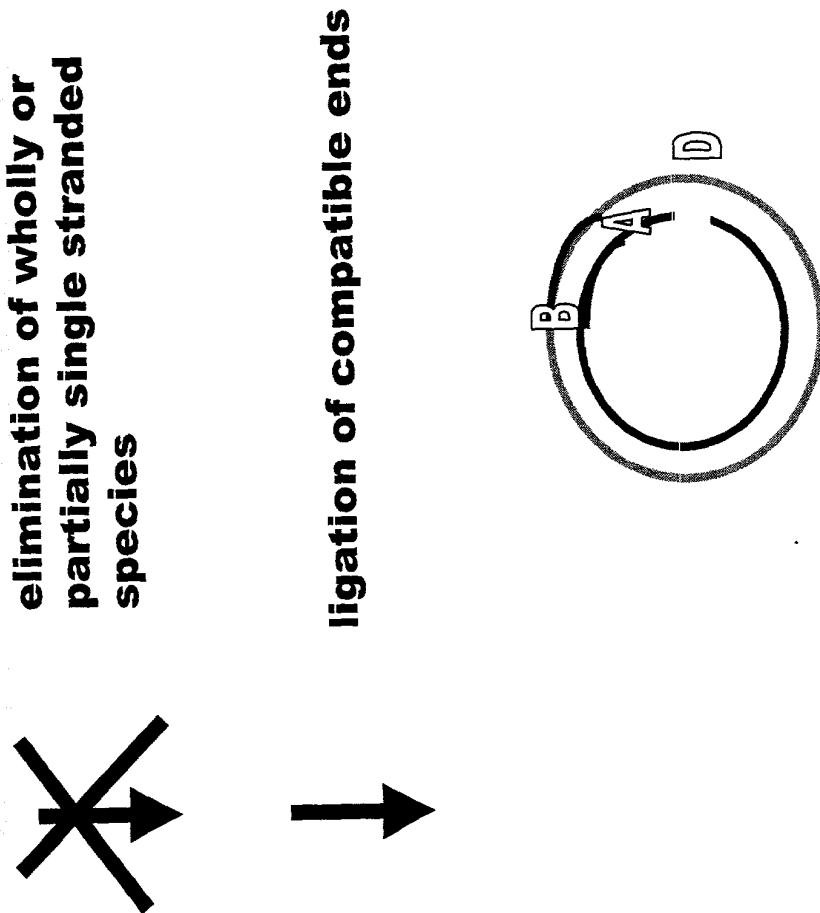
FIG. 9B
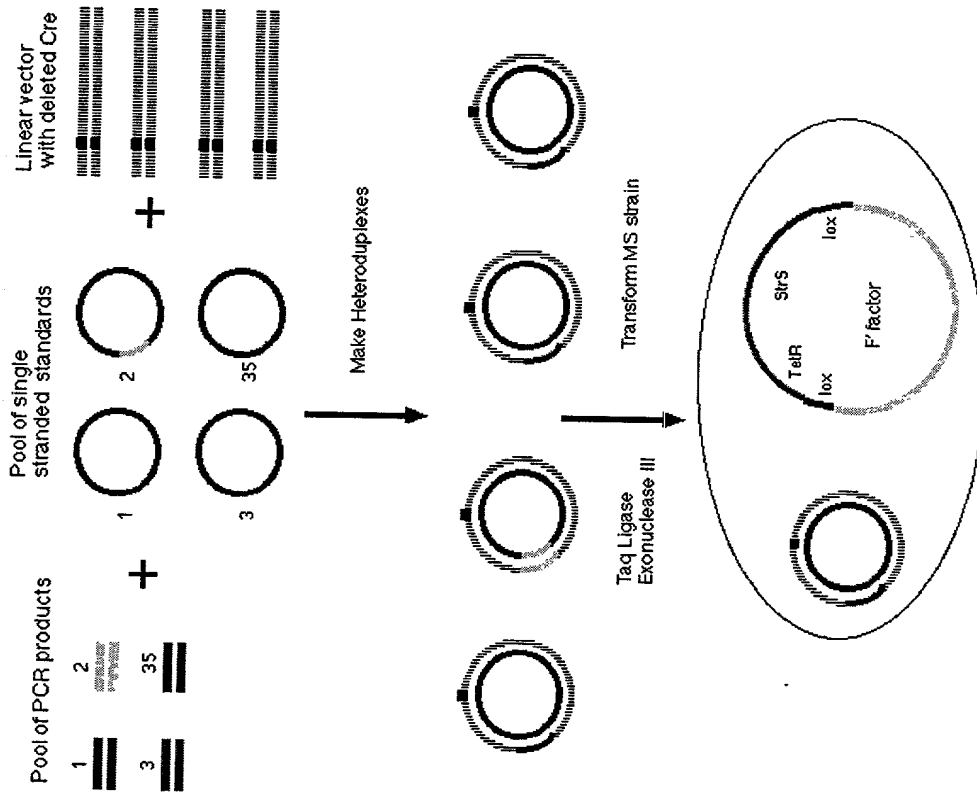
**FIG. 10A**

**FIG. 10B**

FIG. 10C

FIG. 11